JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Low-phytic acid barley improves calcium and phosphorus utilization and growth performance in growing pigs

T. L. Veum, D. R. Ledoux, D. W. Bollinger, V. Raboy and A. Cook

J Anim Sci 2002. 80:2663-2670.

Low-phytic acid barley improves calcium and phosphorus utilization and growth performance in growing pigs[1]

T. L. Veum[2]*, D. R. Ledoux*, D. W. Bollinger*, V. Raboy†, and A. Cook†

*Department of Animal Sciences, University of Missouri, Columbia, 65211-5300; and †USDA-ARS, National Small Grains Germplasm Research Facility, Aberdeen, ID 83210

ABSTRACT: Thirty-five crossbred barrows averaging 13.5 kg starting BW were used in a 35-d experiment to compare the availability of P and the nutritional value of two near-isogenic progeny of the barley cultivar 'Harrington'. Low-phytic acid barley (LPB, 0.35% total P, 0.14% phytic acid P) was homozygous for the *low-phytic acid* 1-1 allele, and the normal barley (NB, 0.35% total P, 0.24% phytic acid P) was homozygous for the normal allele of that gene. Pigs were fed individually twice daily in metabolism pens. Barley was the only source of phytate in semipurified diets, 1 to 3. Diet 1 contained 75% NB, 0.14% estimated available P (aP), and 0.50% Ca. Diet 2 contained 75% LPB, 0.22% aP, and 0.50% Ca. No inorganic P (iP) was added to Diets 1 and 2 in order to measure the animal response to the different concentrations of aP in these cultivars. Diet 3 was NB Diet 1 supplemented with iP to equal the concentration of aP in LPB Diet 2. Practical barley–soybean meal (SBM)-type diets, NB Diet 4 and LPB Diet 5, were formulated to meet all minimum nutrient requirements, and contained 0.30% aP and 0.65% Ca. For the semipurified diets, pigs fed LPB Diet 2 had higher ($P \le 0.05$) bone ash weight, bone breaking strength, P absorption and retention, and Ca absorption and retention compared with pigs fed NB Diet 1, with a trend ($P = 0.10$) for pigs fed LPB Diet 2 to have a higher ADG and gain:feed ratio than pigs fed NB Diet 1. However, pigs fed LPB Diet 2 or NB Diet 3 were not different ($P \ge 0.3$) in growth performance, fresh bone weight, fat-free dry bone weight, bone ash, bone breaking strength, or N utilization. This indicates that LPB and NB were equal in nutritional value after supplementation of NB with iP to equal the estimated aP in LPB. For the practical barley-SBM diets, there were no differences ($P \ge 0.4$) between pigs fed NB Diet 4 or LPB Diet 5 for growth performance, fresh bone weight, bone breaking strength, the percentages of P and Ca utilization, or N, DE, and ME utilization. The use of LPB in pig diets reduced P excretion in swine waste by 55% and 16% in our semipurified and practical diets, respectively, compared with NB. Using our in vitro procedure designed to mimic the digestive system of the pig, the availability of P for pigs was estimated at 52% for LPB and 32% for NB.

Key Words: Barley, Calcium, Phosphorus, Phytic Acid, Pigs

 J. Anim. Sci. 2002. 80:2663–2670

Introduction

About 70% of the P in barley is not utilized by swine because it is bound as phytate (myo-inositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate; Maga, 1982; NRC, 1998; Lott et al., 2000). Swine do not produce adequate quantities of the digestive enzyme phytase required to hydrolyze phytate (Pointillart et al. 1984, 1987). Therefore, swine excrete about 200,000 tons of P annually in the United States because of the poor P digestibilities of cereal grain and oilseed meal-based diets (Cromwell and Coffey, 1991). However, a recombinant-derived microbial phytase (Heinzl, 1996) is effective as a feed additive in hydrolyzing phytate in plant ingredient-based diets fed to swine, and significantly reduces P excretion in swine waste (Cromwell et al., 1995; Liu et al. 1997a, 1998a).

The development of a low-phytic acid corn hybrid by USDA scientists greatly increased the availability of P in corn-based diets fed to nonruminants (Ertl et al., 1998; Raboy et al., 2000), significantly reducing the excretion of P in poultry waste (Li et al., 2000) and swine waste (Spencer et al., 2000; Veum et al., 2001). A low-phytic acid mutant barley has also been devel-

[1]The Idaho Barley Commission, Boise, Idaho, and the Alberta Barley Commission, Calgary, Alberta, Canada, are acknowledged for providing partial funding for this research. Thanks are extended to Amy Miles and John Dorsch for their assistance in the field production and laboratory analysis of the barley cultivars, and to Jessica Smith and Qiang Zhang for their assistance in animal care, sample collection, and laboratory analyses.

[2]Correspondence: 112 Animal Sciences Center (phone: 573-882-4331; fax: 573-882-6827; E-mail: veumt@missouri.edu).

Received August 20, 2001.
Accepted June 26, 2002.

oped by USDA scientists (Larson et al., 1998; Raboy et al., 2001) that has been shown to increase the availability of P for fish (Sugiura et al., 1999) and poultry (Li et al., 2001b), with a significant reduction in the excretion of P in poultry waste (Li et al., 2001a). The objectives of this experiment were to evaluate a low-phytic acid mutant barley (**LPB**) for growing swine compared to the near-isogenic normal barley (**NB**) cultivar, and to determine if the decrease in phytic acid content would change the nutritional value of LPB compared to NB. In addition, an in vitro procedure that simulates the digestive system of the growing pig was used to estimate the availability of P in LPB and NB.

Materials and Methods

Animals and Housing. Thirty-five crossbred Yorkshire-Landrace-Duroc barrows averaging 13.5 ± 0.1 kg and 42 ± 1 d of age were allotted to five treatments in a completely random design for the 35-d experiment. Pigs were placed in individual elevated stainless steel metabolism pens (four pens/treatment with 0.9 m^2/pig and three pens/treatment with 0.7 m^2/pig) equipped with nipple drinkers, stainless steel feeders, and stainless steel woven wire floors. Temperature was maintained at 23 ± 1°C with thermostatically controlled heaters and exhaust fans. Pigs were fed to appetite twice daily (0730 and 1530). Individual feed consumption was determined weekly. Pigs were weighed at the beginning and end (d 0 and 35) of the test. This experiment was approved by the University of Missouri Animal Care and Use Committee.

Dietary Treatments. The LPB and NB cultivars used in this experiment were provided by the National Small Grains Germplasm Research Facility, USDA-ARS, Aberdeen, ID. The NB was the cultivar 'Harrington' and the LPB mutant was a near-isogenic progeny of the barley cultivar 'Harrington'. The LPB isoline was homozygous for the *low-phytic acid* 1-1 allele and produced grain with about 42% less phytic acid than the NB. The NB isoline was homozygous for the wild-type allele of that gene, and produced grain with normal concentrations of phytic acid. Before diet formulation, stocks of the mutant LPB hybrid, the near-isogenic NB, the soybean meal (**SBM**), the whey protein concentrate (**WP**), and the spray-dried blood cells (**BC**) were analyzed in duplicate for proximate analysis components (AOAC, 1990) and complete amino acids according to Benson and Patterson (1971). Samples were hydrolyzed under N with 6 *N* HCl for 24 h at 110°C before amino acid analysis was performed by automated cation exchange chromatography. Analysis for cystine and methionine involved performic acid oxidation prior to hydrolysis. Tryptophan was determined by the method of Spies and Chambers (1949).

Triplicate samples of the above ingredients plus dicalcium phosphate, monosodium phosphate, and ground limestone were digested with a wet ash procedure (AOAC, 1990). Digests were analyzed for the concentration of total P (**tP** ,minus ground limestone) by the colorimetric molybdovanadate method (Spectra Rainbow Microplate Reader, Tecan, Inc., Durham, NC) and for the concentrations of Ca (minus monosodium phosphate) by atomic absorption spectrophotometry (Spector AA-30, Varian Analytical Instruments, San Fernando, CA). Subsamples of both barley cultivars and SBM were analyzed for phytic acid P as described by Raboy et al. (2000). The difference between tP and phytic acid P concentration was defined as calculated **aP**. Subsamples of both barley cultivars were also analyzed for GE by oxygen bomb calorimetry (Parr Instrument Co., Moline, IL). The nutrient composition of the two barley cultivars (Table 1) was similar except for the differences in phytic acid P concentration and calculated aP.

Table 1. Analyzed composition (%) of air-dry barley cultivars and soybean meal[a]

Item	Normal barley	Low-phytate mutant barley	Soybean meal
Nutrient			
Total P	0.35	0.35	0.75
Phytic acid P	0.24	0.14	0.53
Available P	0.11	0.21	0.22
Ca	0.06	0.06	0.40
Lysine	0.41	0.41	3.00
Methionine	0.19	0.18	0.67
Cystine	0.27	0.28	0.79
Tryptophan	0.12	0.12	0.74
Threonine	0.35	0.36	1.82
Isoleucine	0.38	0.39	2.23
Valine	0.54	0.55	2.36
CP	10.70	10.70	48.70
GE, kcal/kg	3,876	3,863	4,335

[a]Analyzed values are based on duplicate or triplicate analyses determined prior to the formulation and mixing of the diets. Available P was calculated by subtracting phytic acid P from total P.

There were five dietary treatments (Table 2). Diet 1 was a NB-WP-BC diet containing 0.14% aP and 0.50% Ca. Diet 2 was a LPB-WP-BC diet containing 0.22% aP and 0.50% Ca. Diet 3 was a NB-WP-BC-inorganic P (**iP**) diet, which was similar to Diet 1, but with monosodium phosphate added to increase the aP to 0.22% to equal the concentration of aP in Diet 2. Diets 1, 2, and 3 were semipurified-type diets, where the barley cultivars were the only source of phytate. Diets 1 and 2 were not supplemented with any iP. The concentrations of aP in Diets 1, 2, and 3 (0.14, 0.22, and 0.22%, respectively) were below the NRC (1998) requirements (0.32% at 10 to 20 kg BW, and 0.23% at 20 to 50 kg BW), which provided a strong test of the barley cultivars as sources of aP. Dietary Ca was also reduced to minimize the negative effects of Ca on P utilization (Liu et al., 1998a). Diets 1 to 3 were formulated to meet or exceed all nutrient requirements except for Ca and P. Practical barley-soybean type diets, NB Diet 4 and LPB Diet 5, were formulated to meet or exceed all nutrient requirements and contained 0.30% aP and 0.65% Ca.

Table 2. Ingredient composition (%) of air-dry diets

Item	Diet no.: Diet description:	1 NB-WP-BC	2 LPB-WP-BC	3 NB-WP–BC-iP	4 NB-SBM	5 LPB-SBM
		Dietary treatment[a]				
Normal barley		75.00	—	75.00	74.40	—
Mutant barley		—	75.00	—	—	74.68
Whey protein concentrate		8.00	8.00	8.00	—	—
Soybean meal, 48%		—	—	—	17.56	17.56
Spray-dried blood cells		2.00	2.00	2.00	—	—
Lactose		5.21	5.22	4.75	—	—
Lard		3.41	3.41	3.57	5.00	4.89
Cellulose		0.50	0.50	0.50	—	—
Monosodium phosphate[b]		—	—	0.30	—	—
Dicalcium phosphate[c]		—	—	—	0.97	0.56
Limestone		0.87	0.87	0.87	0.69	0.92
L-Glutamic acid		3.30	3.30	3.30	—	—
L-Lysine		0.42	0.42	0.42	0.28	0.28
DL-Methionine		0.09	0.09	0.09	0.05	0.06
L-Threonine		0.06	0.05	0.06	—	—
L-Isoleucine		0.09	0.09	0.09	—	—
White salt		0.40	0.40	0.40	0.40	0.40
Vitamin & medication premixes[d]		0.50	0.50	0.50	0.50	0.50
Trace mineral premix[e]		0.15	0.15	0.15	0.15	0.15
Dietary composition[f]						
Ca		0.50	0.50	0.50	0.65	0.65
Total P		0.32	0.32	0.40	0.57	0.50
Phytic acid P		0.18	0.10	0.18	0.27	0.20
Available P		0.14	0.22	0.22	0.30	0.30
CP		15.00	15.00	15.00	16.80	16.83
Lysine		1.05	1.05	1.05	1.05	1.05
Methionine + cystine		0.59	0.60	0.59	0.65	0.66
Tryptophan		0.18	0.18	0.18	0.22	0.22
Threonine		0.58	0.58	0.58	0.58	0.59
Isoleucine		0.55	0.55	0.55	0.67	0.68
ME, Mcal/kg		3.26	3.26	3.26	3.25	3.25

[a]NB = normal barley, LPB = low phytate barley, WP = whey protein concentrate, BC = spray-dried blood cells, iP = inorganic P, and SBM = soybean meal.
[b]Monosodium phosphate contains 25.3% P.
[c]Dicalcium phosphate contains 22.1% Ca and 18.5% P.
[d]Vitamin and medication premixes provided per kilogram of diet: 11,000 IU of vitamin A acetate; 1,100 IU of vitamin D_3; 44.1 IU of vitamin E as DL-α-tocopheryl acetate; 4.0 mg of vitamin K as menadione sodium dimethylpyrimidinol bisulfite complex; 30.3 μg of vitamin B_{12}; 8.3 mg of riboflavin; 28.1 mg of pantothenic acid as D-calcium pantothenate; 33.1 mg of niacin; 551.3 mg of choline as choline chloride; 220.5 μg of biotin; 1.65 mg of folic acid; and 110 mg of tylosin.
[e]Trace mineral premix provided per kilogram of diet: 165 mg of Zn as $ZnSO_4$; 165 mg of Fe as $FeSO_4$; 33 mg of Mn as $MnSO_4$; 16.5 mg of Cu as $CuSO_4$; 0.3 mg of I as $Ca(IO_3)_2$; and 0.3 mg of Se as Na_2SeO_3.
[f]Composition is based on triplicate analyses of each diet except for ME that was obtained from NRC (1998) and available P that was calculated by subtracting phytic P from total P.

Measurements. Diets contained 0.05% chromic oxide as a nondigestible indicator. Fecal grab samples and total urine collections were made twice daily from d 29 to 33. Fecal samples were immediately frozen in plastic freezer bags until analyzed. Urine was collected in plastic pails containing 25 mL of 6 *N* HCl. Total urine volume was recorded, and 10% was saved in 1-L screw-cap plastic bottles that were immediately frozen until analyzed. The 5-d fecal collections for individual pigs were thawed, pooled, and air-dried at 55°C. The dried fecal samples and samples of each diet were ground to pass through a 1.0-mm screen before analysis in triplicate for N, Ca, and P as described for the dietary ingredients. The 5-d complete urine collections for individual pigs were thawed, pooled, mixed, and subsampled before analysis for N, Ca, and P. Subsamples of feces, diet, and urine were also analyzed in triplicate for Cr (AOAC, 1990) by atomic absorption spectrophotometry, and for GE by oxygen bomb calorimetry.

On d 35, the pigs were sacrificed (electrically stunned followed by exsanguination). The right-front foot and elbow were removed and refrigerated at 2°C. The third metacarpal and radius bones were excised and cleaned of all adhering tissue within 3 d for size measurements and the determination of breaking strength and ash weight. A caliper (Model CDS6, Mitutoyo Corp., Japan) was used to measure metacarpal and radius bone length and the midshaft widths at the narrowest and widest points. Breaking strength of the fresh bones was determined using an Instron testing machine (Model TML, Instron Corp., Canton, MA), similarly to the procedure described by Crenshaw (1986). Force was ap-

Table 3. Pig growth performance

Item	Diet no.: / Diet description: / Available P, %:	1 NB-WP-BC 0.14	2 LPB-WP-BC 0.22	3 NB-WP-BC-iP 0.22	4 NB-SBM 0.30	5 LPB-SBM 0.30	SEM
Pig wt, kg							
d 0		13.48	13.46	13.46	13.54	13.45	0.40
d 35[b]		30.34	32.45	33.52	34.28	34.30	1.11
ADG, g[b]		482	543	573	599	596	27
ADFI, g[c]		1,063	1,120	1,160	1,134	1,172	40
Gain/feed ratio, g/kg[b]		454	484	494	525	507	12

Dietary treatment[a]: diets 1–5.

[a]NB = normal barley, LPB = low phytate barley, W*P* = whey protein concentrate, BC = spray-dried blood cells, i*P* = inorganic P, and SBM = soybean meal. Each mean represents seven individually penned pigs.
[b]Diet 1 vs Diet 2 ($P = 0.10$), and Diet 1 vs Diet 3 ($P \leq 0.05$).
[c]Diet 1 vs Diet 3 ($P = 0.10$).

plied to the center of the bone, which was held by two supports spaced 3.0 cm apart. After the determination of breaking strength, the bones were wrapped with cheesecloth, boiled in deionized water for 2 h, dried at 55°C for 24 h, and extracted with ethyl ether for 4 to 5 d. Ash weight was determined after the fat-free bones were dried at 55°C and 100°C for 18 and 2 h, respectively, and ashed in a muffle furnace at 600°C for 16 h (AOAC, 1990).

In Vitro Phosphorus Release Comparison.

Our in vitro digestion procedure, which was developed to predict the availability of P in corn–soybean meal (SBM) diets and feed ingredients was used to estimate the availability of P in LPB and NB (Liu et al., 1997b; 1998b). The procedure was designed to mimic the digestive system of growing swine by using separate peptic and pancreatic digestion periods. Samples of NB and LPB were ground through a 1-mm screen and refrigerated until analyzed. Three replicate subsamples of LPB and NB were analyzed by the in vitro procedure to determine P release. Phosphorus concentration in the supernate was determined colorimetrically by the molybdovanadate method (AOAC, 1990). The availability of P was estimated to be 52% for LPB and 32% for NB by our in vitro digestion procedure.

Statistical Analysis. Data from all five dietary treatments were analyzed by ANOVA as a randomized complete block design with individual pigs as the experimental units (Snedecor and Cochran, 1989) using the statistical procedures of SAS (SAS Inst. Inc., Cary, NC). The preplanned single-df comparisons were Diet 1 vs 2, 1 vs 3, 2 vs 3, and 4 vs 5.

Results

Pig Growth Performance. For semipurified Diets 1 to 3, pigs fed LPB Diet 2 had ADFI, ADG, and gain:feed ratios similar ($P \geq 0.50$) to pigs fed NB Diet 3, which contained monosodium phosphate to equal the aP concentration of LPB Diet 2 (Table 3). There was a trend for pigs fed LPB Diet 2 to have higher ($P = 0.10$) ADG (12.7%) and gain:feed ratios (6.6%) than pigs fed NB Diet 1. Pigs fed NB Diet 3 had higher ($P \leq 0.05$) 35-d BW, ADG, and gain:feed ratios compared with pigs fed NB Diet 1, with a trend for a higher ($P = 0.10$) ADFI. For the practical diets containing SBM and equal concentrations of aP (LPB Diet 4 vs NB Diet 5), there were no differences ($P \geq 0.5$) between pigs fed NB Diet 4 or LPB Diet 5 in growth performance criteria.

Bone Characteristics. For metacarpal bone characteristics (Table 4), pigs fed LPB Diet 2 or NB Diet 3 (semipurified diets equal in aP concentration) were similar ($P \geq 0.4$) in fresh metacarpal weight, fat-free dry weight, ash weight, and breaking strength. Pigs fed LPB Diet 2 or NB Diet 3 had higher fat-free dry weights ($P \leq 0.01$), ash weights ($P \leq 0.01$), and metacarpal breaking strength ($P \leq 0.05$) compared with pigs fed NB Diet 1. For the practical diets, pigs fed LPB Diet 5 tended to have higher ($P < 0.06$) metacarpal fat-free dry weight and ash weight than pigs fed NB Diet 4. However, there were no differences ($P \geq 0.7$) between pigs fed practical NB Diet 4 or practical LPB Diet 5 for metacarpal fresh weight and breaking strength. For metacarpal bone size measurements, there were no treatment differences ($P \geq 0.3$) for any of the preplanned treatment comparisons made (data not presented). The overall means ± SE for bone length and midshaft width at the narrowest and widest points were 58.9 ± 1.0 mm, 12.0 ± 0.3 mm, and 16.2 ± 0.3 mm, respectively.

For radius bone characteristics (Table 4), pigs fed LPB Diet 2 or NB Diet 3 did not differ ($P \geq 0.4$) in radius fresh weight, fat-free dry weight, ash weight, or breaking strength. Pigs fed LPB Diet 2 or NB Diet 3 had higher fat-free dry weights ($P \leq 0.001$), ash weights ($P \leq 0.001$), and radius breaking strength ($P = 0.04$) than pigs fed NB Diet 1. For pigs fed the practical diets, NB Diet 4 or LPB Diet 5, there were no differences ($P \geq 0.5$) for radius fresh weight, fat-free dry weight, ash weight, or breaking strength. For radius bone size measurements, there were no treatment differences ($P \geq 0.2$) for any of the preplanned treatment comparisons made (data not presented). The overall means ± SE for bone length and midshaft width at the narrowest and

Table 4. Metacarpal and radius bone characteristics

Item	Diet no.:	1	2	3	4	5	
	Diet description:	NB-WP-BC	LPB-WP-BC	NB-WP-BC-iP	NB-SBM	LPB-SBM	
	Available P, %:	0.14	0.22	0.22	0.30	0.30	SEM
Metacarpal bone							
Fresh wt, g[b]		11.26	12.46	13.06	12.51	12.57	0.47
Fat-free dry wt, g[c]		2.86	3.41	3.32	3.36	3.81	0.17
Ash wt, g[c]		1.55	1.91	1.82	1.88	2.19	0.10
Breaking strength, kg[d]		32.8	41.6	39.2	46.7	45.2	2.7
Radius bone							
Fresh wt, g[b]		31.89	35.18	37.54	37.44	37.33	1.25
Fat-free dry wt, g[e]		9.15	12.01	11.81	12.89	12.59	0.50
Ash wt, g[e]		4.38	6.07	5.77	6.87	6.52	0.28
Breaking strength, kg[f]		50.1	66.6	66.7	86.9	86.3	5.4

Dietary treatment[a] (columns 1–5)

[a]NB = normal barley, LPB = low-phytate barley, W*P* = whey protein concentrate, BC = spray-dried blood cells, i*P* = inorganic P, and SBM = soybean meal. Each mean represents seven individually penned pigs.
[b]Diet 1 vs Diet 2, and Diet 1 vs Diet 3 ($P \leq 0.07$).
[c]Diet 1 vs Diet 2, and Diet 1 vs Diet 3 ($P \leq 0.01$). Diet 4 vs Diet 5 ($P \leq 0.06$).
[d]Diet 1 vs Diet 2, and Diet 1 vs Diet 3 ($P \leq 0.05$).
[e]Diet 1 vs Diet 2, and Diet 1 vs Diet 3 ($P \leq 0.001$).
[f]Diet 1 vs Diet 2, and Diet 1 vs Diet 3 ($P = 0.04$).

widest points were 92.6 ± 1.3 mm,11.3 ± 0.3 mm, and 18.4 ± 0.4 mm, respectively.

Apparent Phosphorus and Calcium Utilization. Grams of tP consumed per day were higher ($P \leq 0.01$) for pigs fed diets containing NB compared with LPB when both diets were formulated to contain comparable levels of aP (Table 5, NB Diet 3 vs LPB Diet 2, and NB Diet 4 vs LPB Diet 5). Fecal P excretion (g/d) was lower for pigs fed LPB Diet 2 compared with pigs fed NB Diet 3 ($P = 0.0001$) or NB Diet 1 ($P \leq 0.03$). There also was a trend ($P = 0.07$) for pigs fed NB Diet 3 to excrete more grams of urinary P per day than pigs fed NB Diet 1. Pigs fed LPB Diet 2 absorbed and retained more grams of P per day than pigs fed NB Diet 1 ($P = 0.001$) or NB Diet 3 ($P \leq 0.05$). Pigs fed NB Diet 3 absorbed more grams of P per day than pigs fed NB Diet 1 ($P \leq 0.05$), with a trend ($P = 0.08$) for pigs fed NB Diet 3 to retain more grams of P per day than pigs fed NB Diet 1. Expressed as a percentage of intake, P absorption and retention were higher ($P = 0.0001$) for pigs fed LPB Diet 2 compared with pigs fed NB Diet 1 or NB Diet 3, which were not different ($P \geq 0.8$). For the practical diets, pigs fed NB Diet 4 absorbed and retained more grams ($P \leq 0.05$) of P per day than pigs fed LPB Diet 5. However, when P absorption and retention were expressed as a percentage of intake, there were no differences ($P = 0.6$) between pigs fed the NB Diet 4 or the LPB Diet 5.

Calcium intake was not different for pigs fed semipurified Diets 1 to 3 ($P \geq 0.4$) or practical Diet 4 vs 5 ($P = 0.8$) (Table 5). Fecal excretion of Ca (g/d) was lower for pigs fed LPB Diet 2 compared with pigs fed NB Diet 1 ($P = 0.05$) or NB Diet 3 ($P = 0.10$). Urinary excretion of Ca (g/d) was higher for pigs fed NB Diet 1 ($P = 0.01$) or LPB Diet 2 ($P = 0.09$) than pigs fed NB Diet 3. Calcium absorption in grams per day and as a percentage of intake was higher ($P \leq 0.01$) for pigs fed LPB Diet 2 compared with pigs fed NB Diet 1 or NB Diet 3, that were not different ($P \geq 0.4$). Calcium retention (g/d and percentage of intake) was lower ($P \leq 0.001$) for pigs fed NB Diet 1 than pigs fed LPB Diet 2 or NB Diet 3, which were not different ($P \geq 0.3$). For practical NB Diet 4 and LPB Diet 5, there were no differences ($P \geq 0.5$) in Ca absorption, retention, or excretion (fecal and urinary) in grams per day or as a percentage of intake.

Apparent Energy and Nitrogen Utilization. For apparent energy utilization (Table 5), pigs fed LPB Diet 2 excreted fewer ($P \leq 0.05$) kilocalories of energy in feces per day, and had higher percentages ($P \leq 0.05$) of apparent DE (absorbed/intake) and ME (retained/intake) compared with pigs fed NB Diet 3. There were no other preplanned treatment comparison differences ($P \geq 0.4$) for the semipurified Diets 1 to 3 or practical Diet 4 vs 5 for the energy criteria measured.

For apparent N utilization, there were no treatment contrast differences ($P \geq 0.2$) for semipurified Diet 1 vs 2, 1 vs 3, or 2 vs 3, or for practical Diet 4 vs 5 for the N criteria measured (data not presented). The overall means ± SE for N intake, fecal N, urinary N, N absorbed, and N retained in grams per day were 39.2 ± 2.0, 10.1 ± 1.4, 1.7 ± 0.2, 29.1 ± 1.5, and 27.5 ± 1.5, respectively.

Discussion

This in vivo experiment is the first experiment with swine to demonstrate the higher availability of P in mutant LPB compared with the near-isogenic NB cultivar 'Harrington.' Using the same barley cultivars in experiments with turkeys and broiler chicks, Li et al. (2001a,b) have shown that the P in LPB is more available than the P in NB. When chick toe ash was the criteria in a slope-ratio assay (KH_2PO_4 = 100%), the availability of P was 49% for LPB and 28% for NB (Li et al., 2001b). Using our in vitro procedure that mimics

Table 5. Apparent phosphorus, calcium, and energy utilization

Item	Dietary treatment[a]					SEM
Diet no.:	1	2	3	4	5	
Diet description:	NB-WP-BC	LPB-WP-BC	NB-WP-BC-iP	NB-SBM	LPB-SBM	
Available P, %:	0.14	0.22	0.22	0.30	0.30	
ADFI, d 28 to 33, g	1,509	1,534	1,656	1,587	1,628	88
Phosphorus						
Intake, g/d[b]	5.02	5.22	6.81	9.59	7.80	0.40
Fecal, g/d[c]	2.95	1.78	4.03	4.37	3.70	0.31
Absorbed, g/d[d]	2.07	3.44	2.78	5.22	4.10	0.24
Urinary, g/d[e]	0.02	0.06	0.09	0.16	0.10	0.03
Retained, g/d[f]	2.05	3.37	2.69	5.06	4.00	0.24
Absorbed/intake, %[g]	40.48	66.14	41.51	55.14	52.56	3.20
Retained/intake, %[g]	40.10	64.92	40.18	53.31	51.28	3.18
Calcium						
Intake, g/d	7.49	8.13	7.71	9.74	9.89	0.46
Fecal, g/d[h]	2.14	1.33	1.99	3.19	3.05	0.27
Absorbed, g/d[i]	5.35	6.80	5.72	6.55	6.84	0.31
Urinary, g/d[j]	2.90	2.42	1.79	1.06	1.34	0.26
Retained, g/d[k]	2.45	4.38	3.93	5.49	5.49	0.30
Absorbed/intake, %[i]	72.29	83.86	74.41	67.64	69.25	2.42
Retained/intake, %[k]	33.73	54.07	51.16	56.63	55.99	3.34
Energy						
Intake, kcal GE/d	6,144	6,194	6,633	6,606	6,750	361
Fecal, kcal/d[l]	1,249	1,097	1,591	1,341	1,445	178
Absorbed (DE), kcal/d	4,895	5,096	5,042	5,265	5,305	256
Urinary, kcal/d	56	60	60	73	60	9
Retained (ME), kcal/d	4,838	5,037	4,982	5,192	5,245	258
Absorbed/intake, %[l]	79.67	82.27	76.01	79.70	78.59	2.02
Retained/intake, %[l]	78.74	81.32	75.11	78.60	77.70	2.01

[a]NB = normal barley, LPB = low-phytate barley, W*P* = whey protein concentrate, BC = spray-dried blood cells, i*P* = inorganic P, and SBM = soybean meal. Each mean represents seven individually penned pigs.
[b]Diet 1 vs Diet 3, Diet 2 vs Diet 3, and Diet 4 vs Diet 5 ($P \leq 0.01$).
[c]Diet 1 vs Diet 2, and Diet 1 vs Diet 3 ($P \leq 0.03$). Diet 2 vs Diet 3 ($P = 0.0001$).
[d]Diet 1 vs Diet 2 ($P = 0.001$). Diet 1 vs Diet 3, Diet 2 vs Diet 3, and Diet 4 vs Diet 5 ($P \leq 0.05$).
[e]Diet 1 vs Diet 3 $P = 0.07$).
[f]Diet 1 vs Diet 2 ($P = 0.001$). Diet 1 vs Diet 3 ($P = 0.08$). Diet 2 vs Diet 3, and Diet 4 vs Diet 5 ($P \leq 0.05$).
[g]Diet 1 vs Diet 2, and Diet 2 vs Diet 3 ($P = 0.0001$).
[h]Diet 1 vs Diet 2 ($P = 0.05$). Diet 2 vs Diet 3 ($P = 0.10$).
[i]Diet 1 vs Diet 2, and Diet 2 vs Diet 3 ($P \leq 0.01$).
[j]Diet 1 vs Diet 3 ($P = 0.01$). Diet 2 vs Diet 3 ($P = 0.09$).
[k]Diet 1 vs Diet 2, and Diet 1 vs Diet 3 ($P \leq 0.001$).
[l]Diet 2 vs Diet 3 ($P \leq 0.05$).

the digestive system of the pig (Liu et al., 1997b, 1998b), the availability of P was estimated to be 52% for LPB and 32% for NB. The estimated percentage availability of P in NB as determined by the chick in vivo data and the swine in vitro procedure compare favorably with the availability of P estimate of 31% determined analytically for NB by subtracting phytic acid P from tP (0.11% aP/0.35% tP × 100, Table 1). However, the estimated percentage availability of P in LPB as determined by the chick in vivo data and the swine in vitro procedure are lower than the analytical percentage availability of P estimate of 60% for LPB (0.21% aP/0.35% tP × 100, Table 1). The estimated percentage availability of P in LPB is about two times higher than that in NB as determined by analytical analysis.

The barley cultivars were the only source of phytic acid in semipurified Diets 1, 2, and 3 in our in vivo swine experiment. These experiments were designed to test the differences in aP between LPB and NB. Because the source of barley was the only difference between NB Diet 1 and LPB Diet 2, differences between those two treatments may be attributed to the higher aP and lower phytic acid concentration in LPB compared with NB. The NB Diet 3 was Diet 1 supplemented with iP to match the higher concentration of aP in LPB Diet 2. Thus, phytic acid content was the only nutritional difference between Diets 2 and 3, and a comparison of those diets would determine if the decrease in phytic acid concentration in LPB would affect the nutritional value of LPB. Diets 4 and 5 were practical barley–SBM-type diets formulated to meet minimum NRC (1998) requirements. Both diets contained 0.30% aP, with the LPB in Diet 5 providing a greater proportion of the aP compared to the NB in Diet 4.

The increase in pig growth performance, bone ash weight, bone breaking strength, apparent P absorption and retention, and apparent Ca absorption and retention for pigs fed LPB Diet 2 compared with pigs fed NB

Diet 1 confirmed the greater availability of P in LPB. Our results also showed that pigs fed LPB Diet 2 or NB Diet 3, supplemented with iP to equal the estimated aP in LPB Diet 2, had similar growth performance, bone fresh weight and fat-free dry weight, bone ash weight, and bone breaking strength. This indicates that the estimated aP concentrations determined for the LPB and NB cultivars (Table 1) were reasonable, and that the nutritional value of LPB was not compromised because of the lower phytate content compared with NB. In fact, the percentages of apparent DE and ME were higher for pigs fed LPB Diet 2 than NB Diet 3. The endogenous phytase activities of LPB and NB were similar (251 ± 46 and 290 ± 18 U/kg, respectively, Li et al., 2001b), confirming that endogenous phytase activity did not confound the treatment comparisons in this experiment.

Dietary Ca concentration was lowered from 0.65 to 0.50 in semipurified Diets 1 to 3 to keep the Ca:tP ratio below 1.6, because excessive Ca is known to reduce P utilization (Nahapetian and Young, 1980; Liu et al., 1998a, 2000). In this experiment, the increase in growth performance with LPB compared with NB was not as dramatic as in our previous experiment with low-phytic acid corn compared with normal corn (Veum et al., 2001). This may be explained by the fact that the phytic acid concentration was reduced 42% in LPB vs NB in the present experiment, whereas phytic acid concentration was reduced 60% in our low-phytic acid corn relative to our normal corn.

Our practical barley–SBM-type diets (NB Diet 4 and LPB Diet 5) were supplemented with dicalcium phosphate to provide the same adequate (NRC, 1998) concentrations of aP and Ca to determine if LPB would affect growing pig performance. Our results showed that pigs fed practical diets containing NB or LPB were similar in growth performance, bone breaking strength, apparent P and Ca utilization, and apparent N, DE, and ME utilization. Therefore, the results of this experiment indicate that most response criteria for pigs fed diets containing either LPB or NB were similar whether barley was the only source of phytic acid or if SBM was used as a protein supplement when the NB diets contained additional iP to equal the aP in the LPB diets (Diet 2 vs Diet 3, Diet 4 vs Diet 5). This experiment demonstrated that tP and phytic acid P concentrations determined analytically may provide a useful estimate of the aP concentration in barley fed to swine, which has practical application, in diet formulation. The use of analytical data to estimate aP was previously found to have validity in estimating the aP concentration in low-phytic acid and normal corn hybrids (Veum et al., 2001).

The most significant response to feeding LPB was the reduction in P excretion in swine waste. For pigs fed LPB Diet 2 or NB Diet 3, semipurified diets that contained equal concentrations of aP, P excretion (g/d, feces plus urine) was reduced 55% by LPB compared with NB (1.84 vs 4.12 g/d, respectively), which compares favorably with the 50% reduction in P excretion by pigs fed semipurified diets containing low-phytic acid corn compared with normal corn (Veum et al., 2001). For pigs fed the practical barley-SBM diets, P excretion (g/d, feces plus urine) was reduced a less dramatic 16% by LPB compared with NB (3.80 vs 4.53 g/d, respectively). Thus, the phytic acid in SBM had a significant negative effect on P utilization in our practical barley-SBM diets, similar to the negative effect observed in our low-phytic acid corn experiment (Veum et al., 2001). Low-phytic acid soybean mutants have been isolated that have about a threefold increase in the availability of P compared to the normal soybean cultivar, with no reduction in seed tP (Wilcox et al., 2000). The present experiment demonstrates the need for commercial development of a low-phytic acid SBM and/or the use of a phytase enzyme in combination with a low-phytic acid grain to obtain the maximum achievable reduction in P excretion in swine waste when practical grain-SBM diets are fed to growing swine.

In conclusion, the P in LPB was about two times more available than the P in NB as a result of the reduction in phytic acid in LPB. The reduction in phytic acid did not compromise the nutritional value of LPB, and P excretion in swine waste was significantly reduced by feeding LPB. Our in vitro procedure designed to mimic the digestive system of the pig provided a valid estimate for the availability of P in both barley cultivars.

Implications

Feeding a low-phytic acid barley homozygous for the recessive *low-phytic acid* 1-1 allele to swine will greatly reduce the excretion of P in swine waste compared with normal barley. When practical barley-soybean meal diets containing either low-phytic acid barley or normal barley were formulated to contain the same concentration of available P, pig growth performance, bone strength, and utilization of P, Ca, N, digestible energy, and metabolizable energy were similar. Thus, lowering the phytate concentration will not have any adverse nutritional effects on low-phytate barley.

Literature Cited

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Offic. Anal. Chem. Arlington, VA.

Benson, J. V., Jr., and J. A. Patterson. 1971. Chromatographic advances in amino acid and peptide analysis using spherical resins and their applications in biochemistry and medicine. Pages 1–73 in New Techniques in Amino Acid, Peptide and Protein Analysis. A. Niederweiser and G. Pataki, ed. Ann Arbor Sci. Publ., Ann Arbor, MI.

Crenshaw, T. D. 1986. Reliability of dietary Ca and P levels and bone mineral content as predictors of bone mechanical properties at various time periods in growing swine. J. Nutr. 116:2155–2170.

Cromwell, G. L., and R.D. Coffey. 1991. Phosphorus—a key essential nutrient, yet a possible major pollutant—its central role in animal nutrition. Pages 133–145 in Biotechnology in the Feed Industry. T. P. Lyons, ed. Proc. Alltech's 7th Annu. Symp. Alltech Tech. Publ., Nicholasville, KY.

Cromwell, G. L., R. D. Coffey, G. R. Parker, H. J. Monegue, and J. H. Randolph. 1995. Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorus in corn-soybean meal diets for pigs. J. Anim. Sci. 73:2000–2008.

Ertl, D.S., K. A. Young, and V. Raboy. 1998. Plant genetic approaches to phosphorus management in agricultural production. J. Environ. Qual. 27:299–304.

Heinzl, W. 1996. Natuphos® technical specifications. Pages 693–700 in Phytase in Animal Nutrition and Waste Management. M.B. Coelho and E.T. Kornegay, ed. BASF Corp., Mount Olive, NJ.

Larson, S. R., K. A. Young, A. Cook, T. K. Blake, and V. Raboy. 1998. Linkage mapping of two mutations that reduce phytic acid content of barley grain. Theor. Appl. Genet. 97:141–146.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low-phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444–1450.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and K. Zyla. 2001a. Low-phytic acid barley improves performance, bone mineralization, and phosphorus retention in turkey poults. J. Appl. Poult. Res. 10:178–185.

Li, Y.C., D. R. Ledoux, T. L. Veum, V. Raboy, K. Zyla, and A. Wikiera. 2001b. Bioavailability of phosphorus in low-phytic acid barley. J. Appl. Poult. Res. 10:86–91.

Liu, J., D. W. Bollinger, D. R. Ledoux, M. R. Ellersieck, and T. L. Veum. 1997a. Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorus corn-soybean meal diet for growing pigs. J. Anim. Sci. 75:1292–1298.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 1998a. Lowering the dietary calcium to total phosphorus ratio increases phosphorus utilization in low-phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. J. Anim. Sci. 76:808–813.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 2000. Effects of dietary calcium:phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum, and colon of pigs. J. Anim. Sci. 78:106–109.

Liu, J., D. R. Ledoux, and T. L. Veum. 1997b. In vitro procedure for predicting the enzymatic dephosphorylation of phytate in corn-soybean meal diets for growing swine. J. Agric. Food Chem. 45:2612–2617.

Liu, J., D. R. Ledoux, and T. L. Veum. 1998b. In vitro prediction of phosphorus availability in feed ingredients for swine. J. Agric. Food Chem. 46:2678–2681.

Lott, J. N. A., I. Ockenden, V. Raboy, and G. D. Batten. 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Sci. Res. 10:11–33.

Maga, J. A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30:1–9.

Nahapetian, A., and V. R. Young. 1980. Metabolism of ^{14}C-phytate in rats: Effect of low and high dietary calcium intake. J. Nutr. 110:1458–1472.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. Natl. Acad. Press, Washington, DC.

Pointillart, A., N. Fontaine, and M. Thomasset. 1984. Phytate phosphorus utilization and intestinal phytases in pigs fed low phosphorus: wheat or corn diets. Nutr. Rep. Int. 29:473–483.

Pointillart, A., A. Fourdin, and N. Fontaine. 1987. Importance of cereal phytase activity for phytate phosphorus utilization by growing pigs fed diets containing triticale or corn. J. Nutr. 117:907–913.

Raboy, V., P.E. Gerbasi, K. A. Young, S. D. Stoneberg, S. G. Pickett, A. T. Bauman, P. P. N. Murthy, W. F. Sheridan, and D. S. Ertl. 2000. Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. Plant Physiol. 124:355–368.

Raboy, V., K. A. Young, J. A. Dorsch, and A. Cook. 2001. Genetics and breeding of seed phosphorus and phytic acid. J. Plant Physiol. 158:489–497.

Snedecor, G. W., and W. G. Cochran. 1989. Statistical Methods. 8th ed. Iowa State University Press, Ames.

Spencer, J. D., G. L. Allee, and T. E. Sauber. 2000. Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. J. Anim. Sci. 78:675–681.

Spies, J. R., and D. C. Chambers. 1949. Chemical determination of tryptophan in proteins. Procedure N. Anal. Chem. 21:1249–1266.

Sugiura, S.H., V. Raboy, K. A. Young, F. M. Dong, and R. W. Hardy. 1999. Availability of phosphorus and trace minerals in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 170:285–296.

Veum, T. L., D. R. Ledoux, V. Raboy, and D. S. Ertl. 2001. Low-phytic acid corn improves nutrient utilization for growing pigs. J. Anim. Sci. 79:2873–2880.

Wilcox, J. R., G. S. Premachandra, K. A. Young, and V. Raboy. 2000. Isolation of high seed inorganic P, low-phytate soybean mutants. Crop Sci. 40:1601–1605.